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Cytomegalovirus-specific T cell immunity: Design of an innovative functional assay

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Introduction

- **Cytomegalovirus** (CMV) responsible of **10-30 %** of infections in kidney transplant recipients *(Tedesco Silva Junior et al. Transpl Infect Dis. 2023)*
- In **immunocompromised** patients: need to control immune system to avoid **opportunistic infections and graft loss**.
- **Prevention**: **prophylactic treatment**, follow up of CMV replication (**RT-PCR**), and evaluation of cell-mediated immunity (CMI) (**Quantiferon, ELISpot**) *(Rutger Callens et al. Transplant Cell Ther. 2022)*

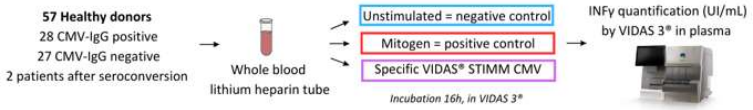
Objective

- **Problematic**: Existing cellular diagnostic tools are very cumbersome and/or are **limited to CD8+ T cell response monitoring**.
- Need new assays (simpler, more automated and robust) to evaluate CMV-specific T cell and to **stratify** individual risk of CMV disease and then **personalized prophylaxis**

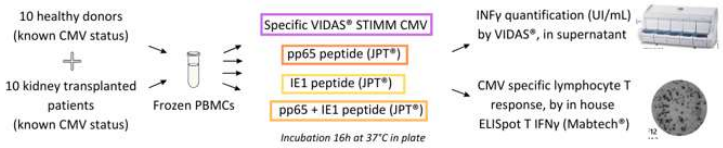
Evaluation of a new Interferon Gamma Release Assay (IGRA) on VIDAS ®. A standardized and fully automated solution to facilitate quantification of CMI response to CMV on VIDAS®.

Method

1/ Workflow and analytical validation on fresh whole blood on healthy volunteers



2/ VIDAS® IGRA vs *in house* ELISpot T IFNγ, on PBMC (at Grenoble University Hospital)



*** VIDAS® STIMM™ CMV RUO:** Multiple CMV antigens at low concentration + immuno-booster designed to improved performances on immunocompromised patients

Results

1/ VIDAS® STIMM™ CMV RUO: Correlation between CMV T cell immunity and serology

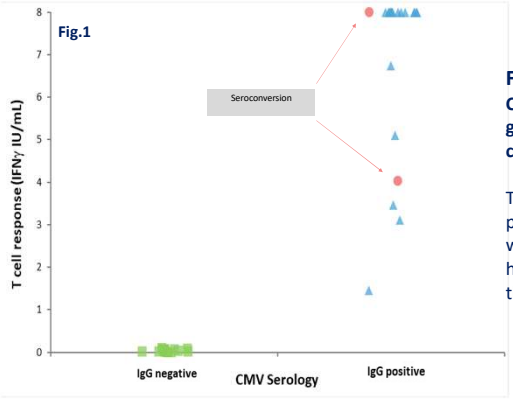


Fig.1- VIDAS® STIMM™ CMV RUO presents a very good specificity and a good correlation with serology.

The two seroconverted patients were found positive with the IGRA test, highlighting the sensitivity of the technic.

2/ VIDAS® STIMM™ CMV RUO : Impact of the formulation

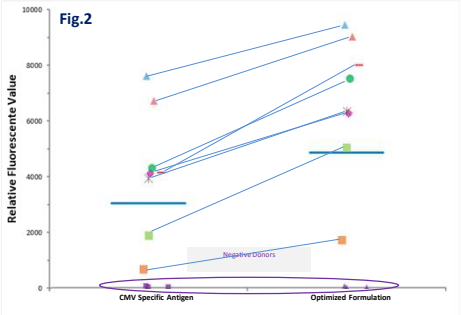
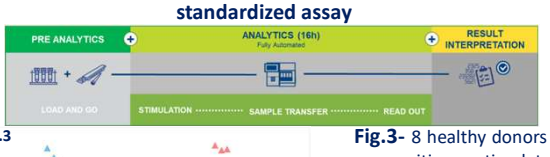


Fig.2- Whole blood of 3 CMV-IgG negative and 8 CMV-IgG positive stimulated with our CMV-specific antigens with or without optimized formulation.

→ **Optimized formulation allows to increase specifically IFN-γ production by CMV-specific T cells**

3/ VIDAS® STIMM™ CMV RUO-IGRA : Towards a fully automated and standardized assay



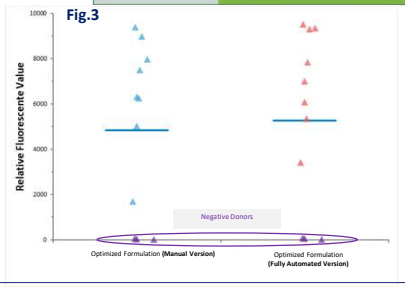


Fig.3- 8 healthy donors CMV-IgG seropositive stimulated with VIDAS® STIMM™ CMV RUO optimized formulation in manual or fully automated version on VIDAS®3.

The fully automated version of the IGRA test contributes to high sensitivity in keeping good specificity

4/ *In house* ELISpot T IFNγ vs CMV-IGRA response on PBMC

- Positive ELISpot T result: >10 spots per well (250 000 cells)
- Positive VIDAS® CMV-IGRA result: IFNγ > 0,10 IU/mL and NIL < 0,08 IU/mL.

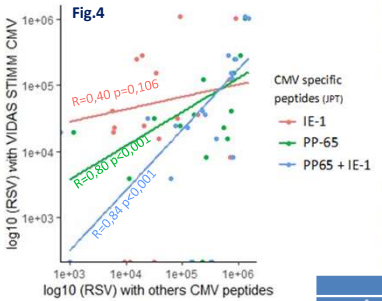


Fig.4- Comparison of PBMC ELISpot T IFNγ response with VIDAS® STIMM™ CMV RUO and others CMV (JPT) T-cell activated peptides.
Better correlation (R=0,84) were obtained with the JPT **pool pp65 + IE-1**

Tab.1- Comparison of qualitative results between VIDAS® STIMM™ CMV RUO (fluorescence value) vs ELISpot T IFNγ (RSV)
Better concordance when using PP-65 or JPT pool (pp65 + IE-1)

Tab.1

	PP65	PP65 + IE-1 (JPT)	IE-1	STIMM™ CMV RUO (bMx)
VIDAS® vs ELISpot	0,95	0,86	0,77	0,68

Conclusion

- **A fully automated and standardized VIDAS® CMV-IGRA assay** to detect CMV-specific cell mediated immunity in whole blood has been developed
- **A sensitive and specific detection of cellular immune response to CMV in immunocompromised patients**, with a unique CMV-specific antigen formulation BMX®
- Activation by VIDAS® STIMM™ CMV RUO correlate with stimulation by pp65 + IE-1 (JPT)
- **Excellent concordance of CMV-IGRA assay** (VIDAS® STIMM™ CMV RUO) and ***in house* ELISpot** with pp65 (JPT)